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Taxonomy and Fine Structure of a Marine Diatom *Achnanthes grunowii*, nom. nov.

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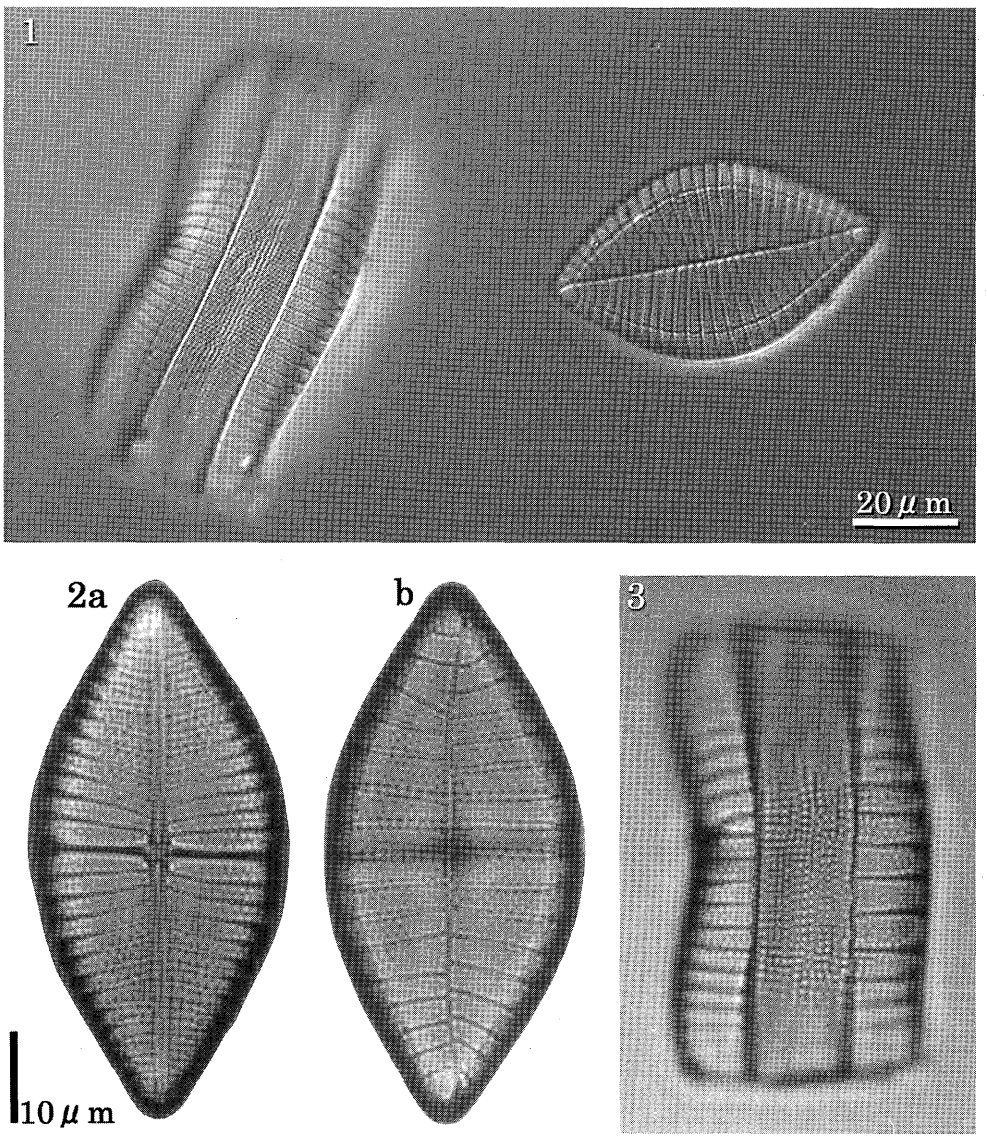
Achnanthes grunowii Toyoda & D. M. Williams was first described by Grunow in Cleve and Grunow (1880), as a variety of *A. javanica* Grunow, *A. javanica* var. *rhombica*. It is shown that the valve face of this taxon is rhombic in shape, and there are marginal and terminal ridges as well as terminal orbiculi on the ARV. The striae stand in more than three rows between costae on the valve, and the areolae stand in more than four rows on the copulae. In contrast, *A. javanica* var. *javanica* has the marginal ridge but no terminal spine, and terminal orbiculi and striae stand in two rows on the valve and small areolae stand in two rows on the valvocopula. These features support the view that *A. javanica* var. *rhombica* should be considered an independent species. However, the specific epithet *rhombica* was used by Østrup (1910) and Meister (1937) in this genus. Hence the new specific epithet *grunowii* is selected.

Key words: *Achnanthes grunowii*, *Achnanthes javanica* var. *rhombica*, *Achnanthes* sensu stricto, nomen novum.

The genus *Achnanthes* was first established by Bory (1822). They are monoraphid, heterovalvar and epiphytic diatoms which curve in the centre of the girdle view. VanLandingham (1967) was able to list more than 500 species in *Achnanthes* sensu lato. The genus has received some attention and been discussed in terms of its relationships with the other monoraphid diatoms by some researchers (cf. Hustedt 1933, Simonsen 1979, Round et al. 1990, Cox and Williams 2004), and has recently been revised to give several new genera (cf. Round et al. 1990, Round and Bukhtiyarova 1996, Round and Basson 1997, Bukhtiyarova and Round 1996). The species in these new genera mostly occur in freshwater, whereas *Achnanthes* sensu stricto occurs in marine and brackish waters, and is usually epiphytic on filamentous seaweeds or other diatom species. The marine species have received considerably less attention than the freshwater species, even though the marine species occur globally.

This study presents new information on the morphology of a marine species from Japan, *Achnanthes grunowii* Toyoda & D. M. Williams, which was originally described as *A. javanica* Grunow var. *rhombica* Grunow (1880). Hustedt (1937) described some figures of this taxon; valve face is rhombic in shape, and the striae stand in

more than two rows between costae. Hustedt's study matches Grunow's description, but lacks taxonomic clarity. Furthermore, fine structure of the frustules have not been revealed using the scanning electron microscope (SEM) until now. As for *A. javanica*, a number of authors misidentified its valve shape, hence the valve shape of this taxon had been described as diverse. Now we know the fine frustule characters of this taxon (see Toyoda et al. 2003), we should reconsider the taxonomic position of *A. javanica* var. *rhombica* based on fine morphological structures eviedent under LM and



Figs. 1–3. Light micrographs of *Achnanthes javanica* (var. *rhombica*). Fig. 1. Hustedt collection (MA/95). Fig. 2. RV (a) and ARV (b) views of the same individual. Fig. 3. Girdle view showing the convex ARV and the concave RV.

SEM.

Materials and Methods

Grunow typified syntype slides of *Achnanthes javanica* var. *rhombica* with Cleve and Möller, No. 147 (see Cleve and Grunow 1880), which were collected from Java. However, the specimens that corresponded to the original description have not been observed in this slide (see Toyoda et al. 2003; Figs. 1–4). Therefore, in order to identify of this taxon correctly, Hustedt's slide (Alfred-Wegener-Institute for Polar and Marine, Germany; No. MA1/95), described in Hustedt in Schmidt (1937), was observed, as Hustedt's description of this taxon corresponds closely to the original, and the material was collected from Miang Basar Borneo, which is the same region as Cleve & Grunow's slide with No. 147.

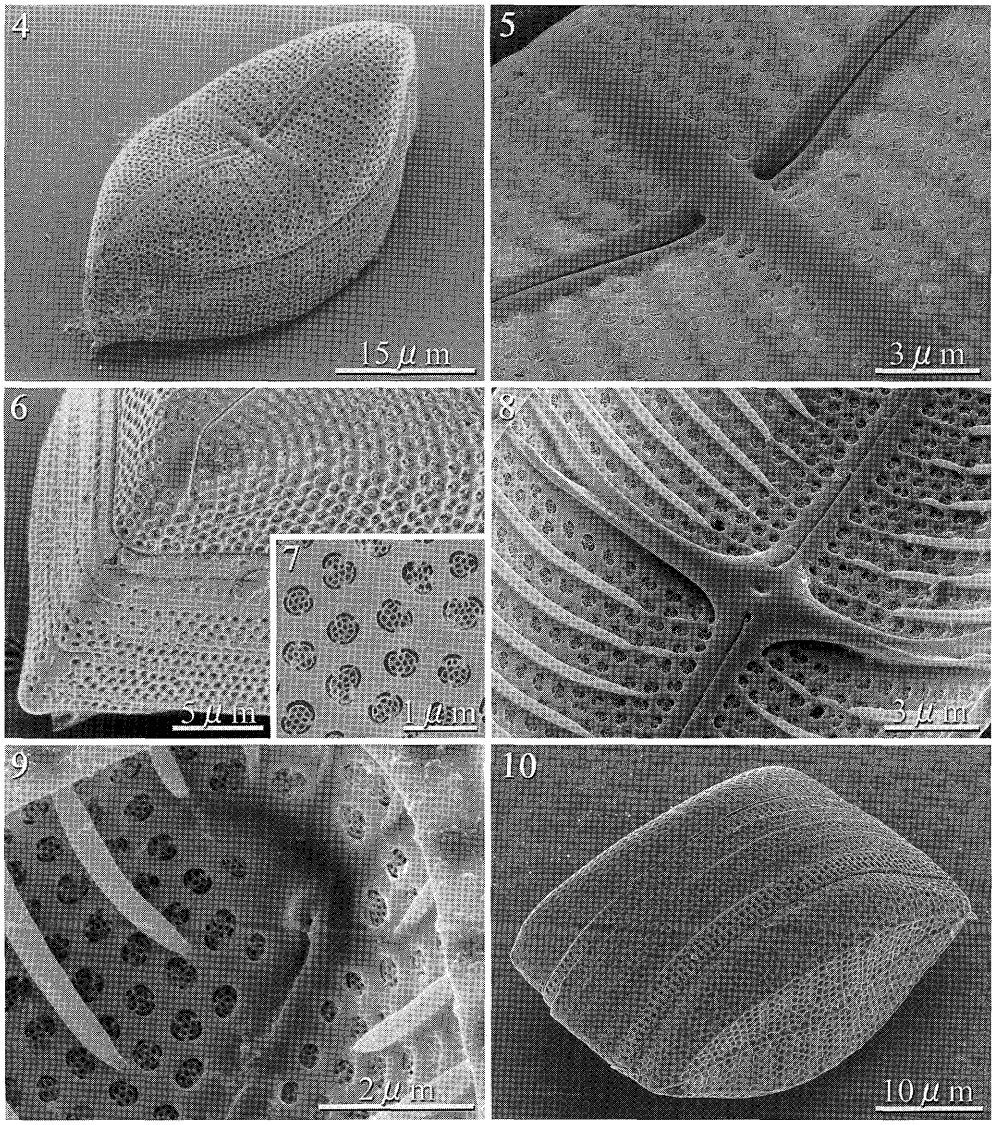
The samples of *A. javanica* var. *rhombica*, that corresponded to the original description were obtained from seaweeds collected from the coast of Japan at the following localities: 1) Kamomejima (41°51′N, 140°09′E), Hokkaido, marine, epiphytic to blue-green algae, 10 Aug. 2001, Coll. A. Kobayashi No. KT1155, 2) Amatsu-Kominato (35°07′N, 140°11′E), Chiba, marine, epiphytic to *Enteromorpha intestinalis* (Chlorophyceae), 15 Dec. 2000, Coll. Toyoda No. KT1125. The numbers refer to the collection housed in Department of Ocean Science, Tokyo University of Marine Science & Technology.

In the Japanese samples, living cells were first observed to identify the plastid morphology using light microscopy (LM). Fresh material was then prepared for LM examination using the bleaching method to prevent the frustule from breaking apart (cf. Nagumo and Kobayasi 1990). In addition, in order to avoid the possibility of mis-identification when only one side of a frustule was observed, the raphid valve (RV) and araphid valve (ARV) faces of the same individuals were examined using a double-faced slide with two coverslips. For SEM observations, cleaned material was dried onto glass cover slips and coated with platinum-palladium, using a HITACHI S-4000 microscope. Valve terminology follows the Working Party on Diatom Terminology (Anonymous 1975, Ross et al. 1979) with supplementary details from Toyoda et al. (2003).

Observation

LM observations of Hustedt's slide (Fig. 1)—The slide includes two individuals, one is ARV faced and the other is girdle view. Valves are rhombic in shape, 66 (89) μm in length and 35 μm in width. On the ARV, the striae stand in more than three rows between costae, transapical costae 6 in 10 μm. There are terminal orbiculi on both ends of the ARV. The rapheless sternum on the ARV is formed almost at the centre.

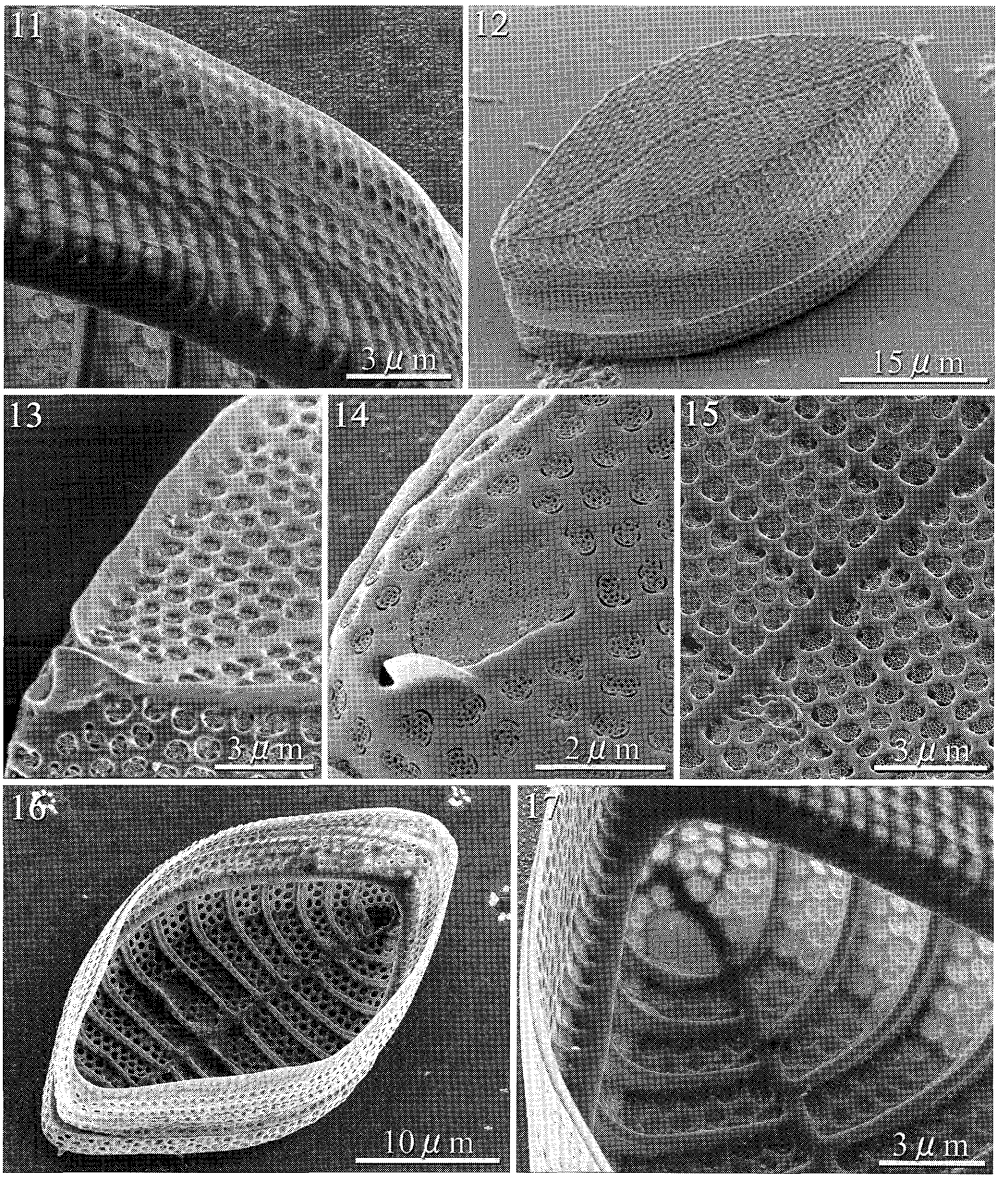
LM observations of Japanese samples (Figs. 2–3)—Valves are rhombic in shape, gently acute at the end of the valve. However, when the frustule is large, valves are spindle-shaped; 33–101 μm in length and 17–32 μm in width. The striae usually stand in two lines between costae on the RV, and in more than four rows on the ARV; transapical costae 6–7 in 10 μm on the RV and 3–5 on the ARV. Frustules usually form a chain of cells attaching to a substratum, which are often thin filamentous seaweeds. They attach to a substratum by a stalk, which is excreted from the one side or both sides of the terminal fissure on the RV. There are two large plastids on either side of the median transapical plane. However, the shape of plastid is variable depending on the status of the individuals. For instance, when the individuals cling to some substratum and form a chain of cells, the inside of the frustule fills with the plastids. On the other hand, when the individuals are moving by circulation of the mucus from the raphe fissure, the plastids move toward the both end of the valve.



Figs. 4–10. SEM micrographs of *Achnanthes rhombica*. Fig. 4. External view of RV. Fig. 5. The central area, stauros showing outer raphe fissures with central pores. Fig. 6. Terminal fissure, curved in the opposite direction to the central pores. Fig. 7. The areolae which are occluded by complex cribrae. Fig. 8. Internal view of central area, stauros. Fig. 9. Internal terminal fissure, curved. Fig. 10. External view of girdles and ARV.

These changes in plastids can also be applied to other species of *Achnanthes*, such as *A. subconstricta*, *A. brevipes* var. *intermedia* and *A. yaquinensis* (cf. Toyoda et al. 2003, Toyoda pers. obs.). The stauros reaches to the margin of the RV. The rapheless sternum on the ARV is formed almost at the centre, and there are terminal orbiculi at the both ends of the ARV.

SEM observations (Figs. 4–17)—Surface of the RV is round (Fig. 4). The raphe fissures have distinctive slightly deflected pores at the centre and the terminal fissures deflect to the opposite side of the direction of the pores (Figs 5–6). An internal view of the



Figs. 11–17. SEM micrographs of *Achnanthes rhombica*. Fig. 11. Internal views of valvocopula and copula showing areolae forming more than four lines. Fig. 12. ARV showing no marginal ridge. Fig. 13. Terminal view of ARV showing marginal ridge with terminal spine. Fig. 14. Detail of the structure of terminal orbiculi. Fig. 15. External view of rapheless sternum with costae. Fig. 16. Internal view of ARV with valvocopula. Fig. 17. Internal view of terminal of ARV showing rim of valvocopula grows inside of the valve.

RV shows that the stauros and costae extend from the raphe sternum and protrude towards the inside (Fig. 8). The raphe fissures are almost straight, but expand slightly at the centre to the same side (Fig. 8), and the terminal fissures curve slightly in the opposite direction of the raphe fissures (Fig. 9). The areolae are occluded by complex cribrae, often supported by three or four pegs and slightly indented, as seen in external views (Fig. 7). The frustule consists of the RV, the ARV and more than three girdle bands (Fig. 10). All girdle bands open at one end with more or less round areolae occluded by cribra; striae in more than four rows, and its rim at the valve side of valvocopula extends inside of the valve (Fig. 11). The ARV face is quite flat when compared with the RV (Fig. 12). Usually, the ARV has marginal ridges and two terminal spines, but occasionally lacks a marginal ridge (Fig. 13). The marginal ridge has a smooth edge, and the terminal spines extend from the marginal ridge at the terminal end of the ARV. The terminal orbiculi are overlaid with a large sort of vola (Fig. 14). The areolae, which are osculated by cribra, occur in more than four rows, and the transverse costae grow from the rapheless sternum unlilaterally (Fig. 15). In internal view of the ARV, costae protrudes towards the inside as on the RV, and extend from the rapheless sternum (Fig. 16). The rim of the valvocopula of the ARV extends inside the valve (Fig. 17).

Discussion

These observations correspond to Grunow's description in respect of the rhombic shape of valve face, frustule size and number of striae. The structure of this taxon shows several different features when compared with *Achnanthes javanica*; the valve face is rhombic in shape, a marginal ridge and terminal spines grow on the ARV, the terminal orbiculi extends to the end of the ARV, more striae occur in between costae on the RV, the ARV and the copulae. In *A. javanica* var. *javanica*, the valve face is spindle-shaped, only the marginal ridge extends to the rim of the ARV, and two small areolae, which are occluded by cribrae, occur in two rows. The marginal ridge and terminal spines can connect on opposed valve faces forming a chain colony (see Toyoda et al. 2003). The type of the formation of the colony is very important for the survival of diatoms in nature, thus, the valve structure is helpful for taxonomic identification in this genus. For example, *A. inflata* Grunow also has only marginal ridges, no terminal spines or marginal special spines as in *A. javanica* (see Le Cohu 1989). *Achnanthes yaquinensis* has two terminal spines, which extend from the rapheless sternum at both end of the valve (Toyoda pers. obs.). *Achnanthes subconstricta* forms a marginal ridge and six marginal spines which have almost the same structure as *A. javanica* var. *rhombica*, the only differences being the number and size of the marginal spines and the size of the marginal ridge. All of these species form chain colonies. *Achnanthes brevipes* var. *intermedia* (Kützing) Cleve does not have any ridges nor spines, it usually forms a chain colonies with less than five individuals (Toyoda per. obs). In addition, the number of rows and the shape of areolae on the copulae are also identifiable features in this genus. The shape of areolae is one the most important taxonomic structures to separate the principal members of *Achnanthes* sensu stricto and from *Achnanthes* sensu lato (cf. Round et al. 1990); that is, only *Achnanthes* sensu stricto species have areolae occluded with a cribrate plate. Others usually have poroid areolae occluded by hymens. The shape of areola usually varies in the species of this genus. Novarino (1992) described the structure of girdles of *A. longipes* Agardh, which has different shapes of areolae in two different rows. *Achnanthes javanica* has small areolae which stand in two rows on the

valvocopula. In *A. yaquinensis*, two areolae lines of different shapes are found on the open bands of the valvocopula; as for normal bands, may be open, with uniform puncta forming two or more rows (Toyoda pers. obs.). It may be possible to identify species in this genus using these band structures.

These results support the view that *A. javanica* var. *rhombica* is best considered a species rather than a variety of *A. javanica*. However, the specific name *A. rhombica* was used by Østrup (1910) and Meister (1935) in this genus. Hence a new specific epithet has been given; *A. grunowii* (see Greuter et al. 2000; the International Code of Botanical Nomenclature, Art. 11 and 23).

Achnanthes grunowii Toyoda & D. M. Williams, nom. nov.

Achnanthes javanica Grunow var. *rhombica* Grunow in Cleve & Möller, Diatoms 4 (1978) – Cleve & Grunow, Kong. Sven. Veten. Hand. 18 (1880); non *Achnanthes rhombica* Østrup (1910); nec *Achnanthes rhombica* Voigt in Meister (1935).

Syntype slide: Cleve & Möller No. 147 (1878, BM 12891).

Type locality: Java, Indonesia.

Achnanthes grunowii has more structural similarities to *A. subconstricta* than *A. javanica* var. *javanica*: Marginal ridges and marginal spines occur on the ARV and form chain colonies, there are terminal orbiculi at the end of valve, and the areolae occur in more than three rows on the copulae. There are, however, several differences between *A. rhombica* and *A. subconstricta* in the valve. In *A. subconstricta*, the outline of the valve face is punduriform, and usually constricted at the centre of the valve. Marginal spines, which usually grow from terminal of marginal ridge, also occur on other parts of the margin, and these are larger when compared with the spines and ridges of *A. rhombica*. In a new systematic study of available data, Cox and Williams (2004) presented the phylogenetic relationships of some Naviculoid diatoms using a cladistic analysis of several morphological characters, including structural data from the genus *Achnanthes*. They suggested that the genus *Achnanthes* has closer relations to *Mastogloia* Thwaites ex W. Smith and *Gomphonema* Ehrenberg than to *Cocconeis* Ehrenberg and *Achnanthes* sensu lato. *Cocconeis* and *Achnanthes* sunsu lato, such as *Achnanthidium* Kützing, which had previously been thought of as closer to between *Achnanthes* sensu stricto (cf. Hustedt 1933, Cleve 1895, Mann 1982). To observe more species of *Achnanthes* sensu stricto from several perspectives, like valve feature, shape of plastid, colony type and ecological syndrome, may change the general idea of systematics of monoraphid diatoms in the future. Study of the rapheless sternum system (e. g., Andrews 1981, Mayama and Kobayasi 1989), has indicated the possibility that *Achnanthes* is derived from biraphid diatoms.

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豊田健介[a,c]，南雲 保[b]，田中次郎[c]，D. M. ウィリアムス[a]：**海産珪藻 *Achnanthes grunowii* の分類および微細構造**

ここでいう，海産付着性珪藻の *Achnanthes grunowii* は，Grunow により1880年に *A. javanica* var. *rhombica* として記載された．本種を本邦より採集し，その微細構造を明らかにした．その結果，殻面は斜方形であり，無縦溝殻に張り出た肋と連結肋及び胞紋域が観察された．これらは *A. javanica* では形成されず，両者は大きく異なった形態をもつことが明らかとなり，本種を独立種と認めた．*Achnanthes* 属ではすでに先行名 *A. rhombica* Østrup (1910) と *A. rhombica* Voigt (1935) があるため，本種を *A. grunowii* と新たに命名した．

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